

## Determining Possible Fragments Based On Experimental Restrictions

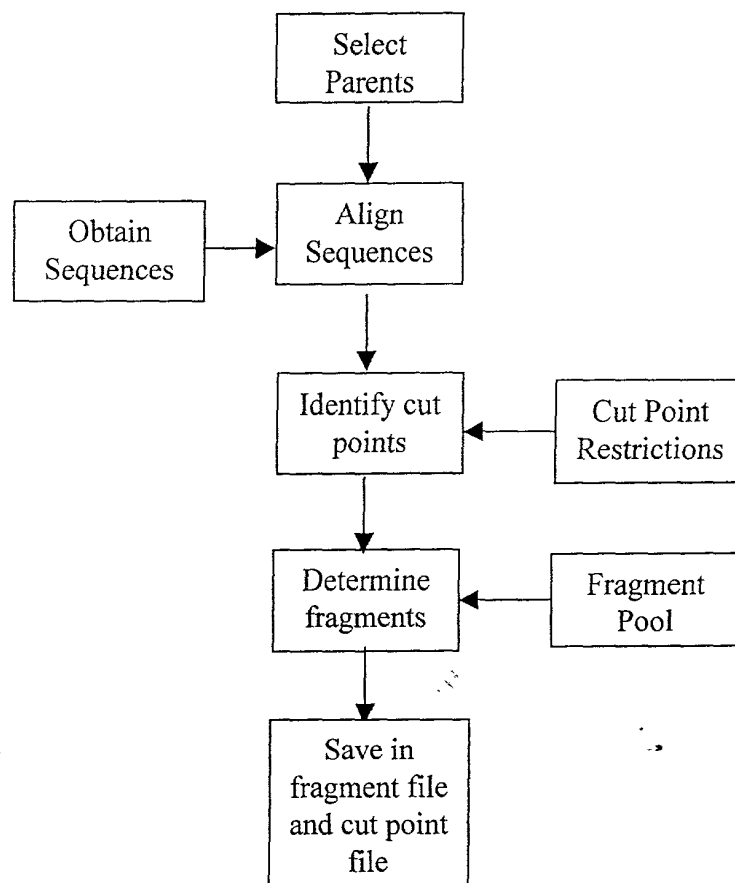


FIG. 1A

## Determining the Schema Disruption Profile for a Structure

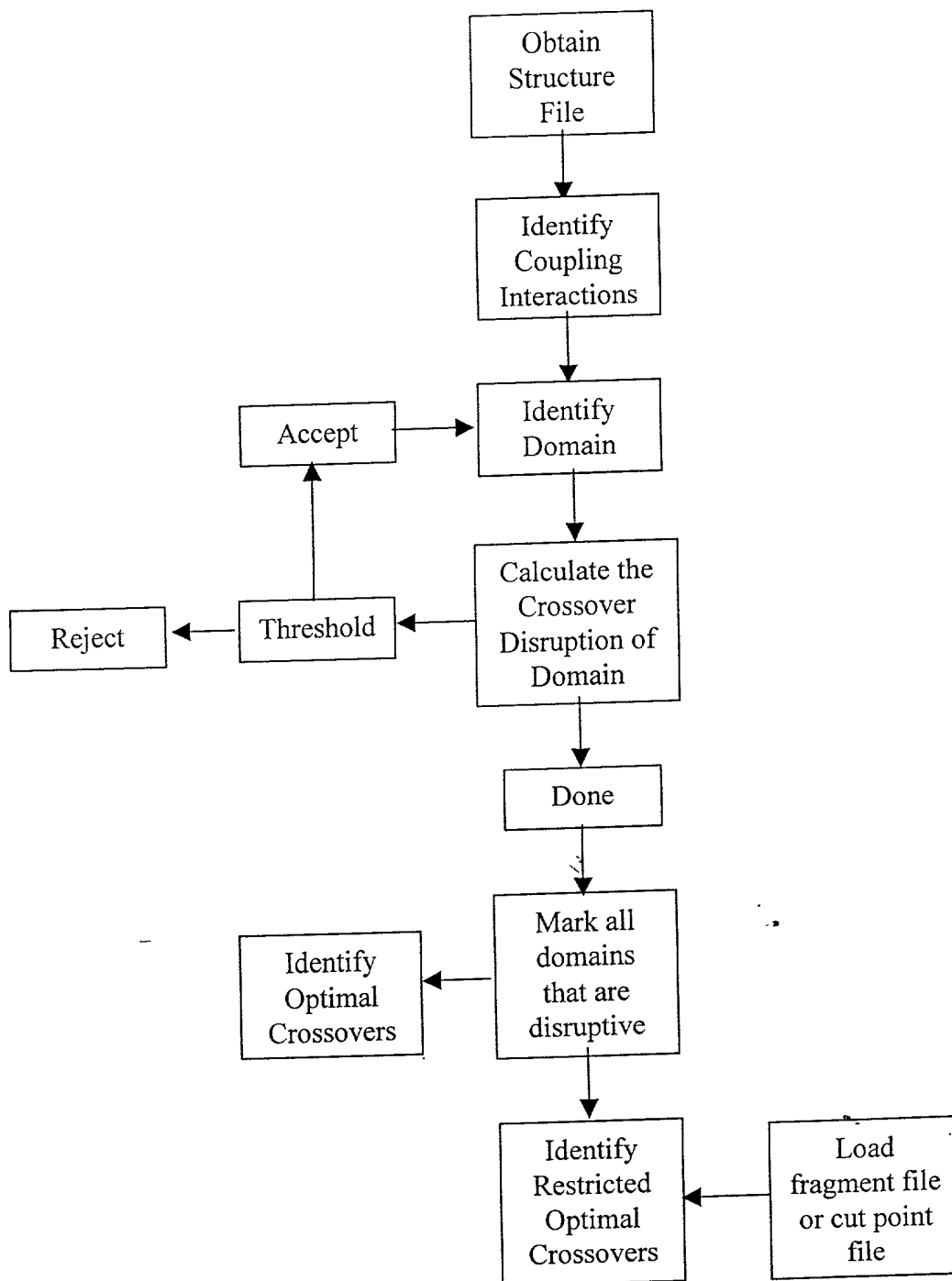


FIG. 1B

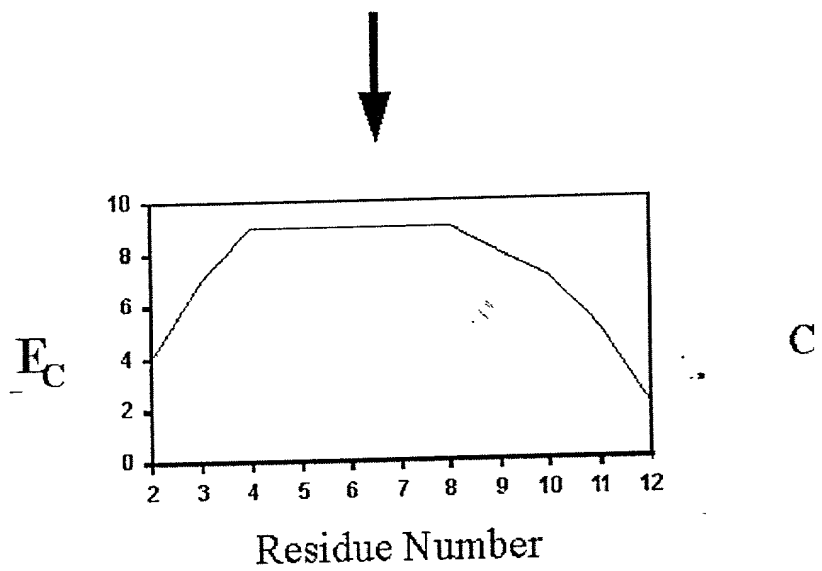
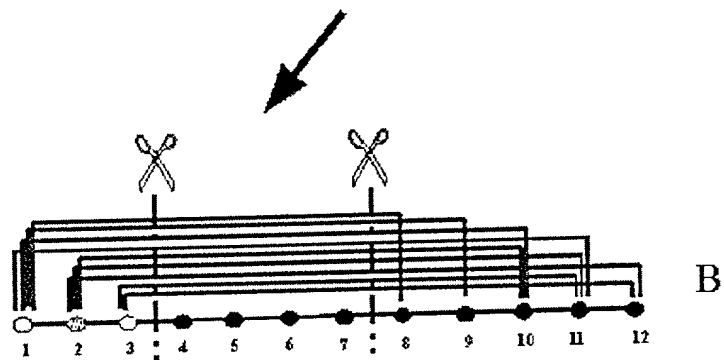
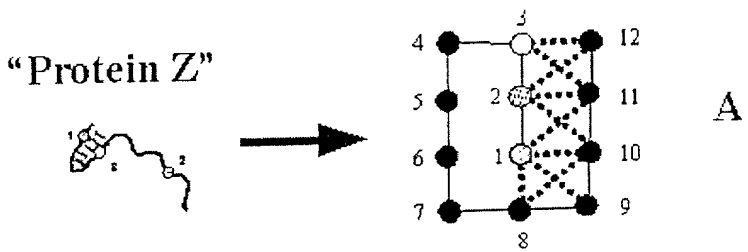
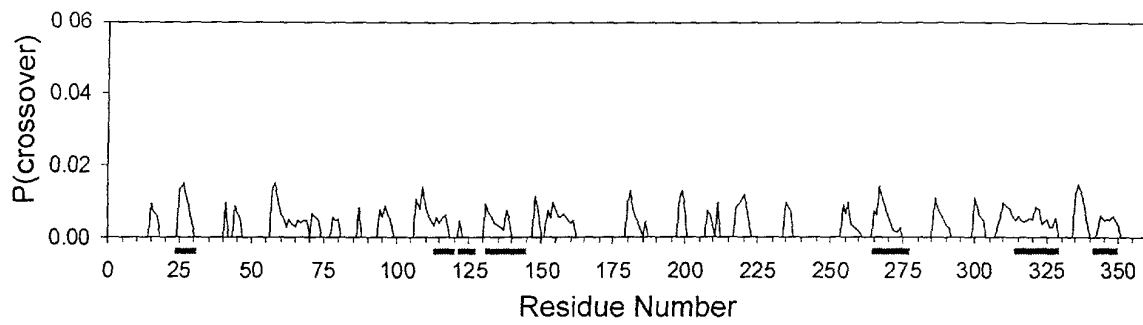


FIG. 2

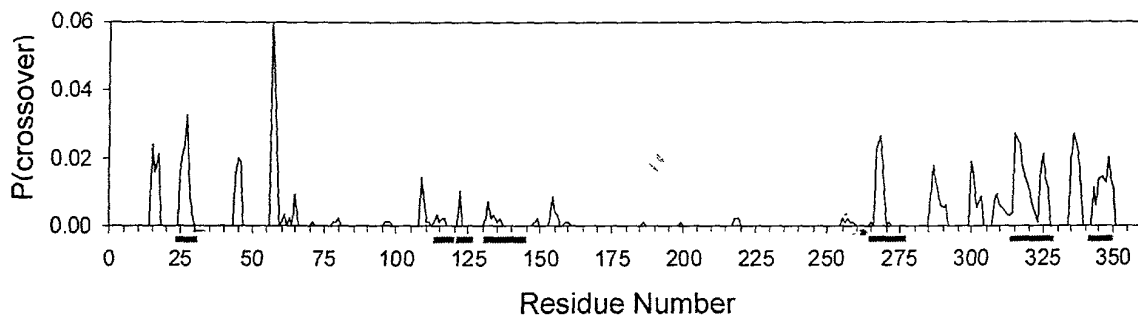
1	1	T	P	V	S	E	E	K	Q	L	A	E	V	A	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A		
2	1	A	K	L	T	E	E	Q	Q	I	A	D	I	V	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A	
3	1	T	K	L	T	E	E	L	Q	V	A	T	I	V	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A	
4	1	Y	A	R	G	E	A	P	L	T	A	A	V	A	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A	
1	51	N	K	P	V	T	P	Q	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q		
2	51	N	H	P	V	T	P	Q	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q		
3	51	G	Q	R	P	V	T	E	N	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
4	51	G	Q	R	P	V	T	E	N	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
1	101	W	Q	G	I	R	S	M	L	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y
2	101	W	R	G	I	R	S	M	L	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y
3	101	W	K	D	V	K	M	L	H	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y		
4	101	F	D	G	V	T	M	A	E	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y		
1	151	A	N	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A			
2	151	A	N	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A			
3	151	S	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
4	151	S	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
1	201	W	G	Y	R	D	G	K	A	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	D	A	S	H	V	Q	E	K	T	L	Q	Q		
2	201	W	G	Y	L	E	G	K	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	D	A	S	H	V	Q	E	K	T	L	Q	Q		
3	201	W	G	Y	K	D	G	Q	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	D	A	S	H	V	Q	E	K	T	L	Q	Q		
4	201	Y	G	Y	K	E	D	K	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	D	A	S	H	V	Q	E	K	T	L	Q	Q		
1	251	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E			
2	251	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E			
3	251	R	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E			
4	249	R	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E			
1	301	V	N	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	N	L	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V				
2	301	V	N	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	N	L	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V				
3	301	L	V	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	N	L	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V				
4	299	F	A	V	P	K	A	M	G	E	Q	R	L	Y	N	K	T	G	S	T	G	G	F	G	A	Y	V	A	F	I	P	E	K	N	L	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V		
1	351	E	A	A	Y	H	I	L	E	A	L	Q																																									
2	351	K	A	A	W	R	I	L	E	K	L	Q																																									
3	351	Q	A	A	Y	D	I	L	Q	A	L	R																																									
4	349	K	A	A	Y	D	I	L	Q	A	L	R																																									

FIG. 3

- |   |                         |                 |
|---|-------------------------|-----------------|
| 1 | Enterbacter cloacae     | P05364 (X03866) |
| 2 | Citrobacter freundii    | P05193 (X07274) |
| 3 | Yersinia enterocolitica | P45460 (X63149) |
| 4 | Klebsiella pneumoniae   | Q48437 (X77455) |



**FIG. 4A**



**FIG. 4B**

4003666-103604

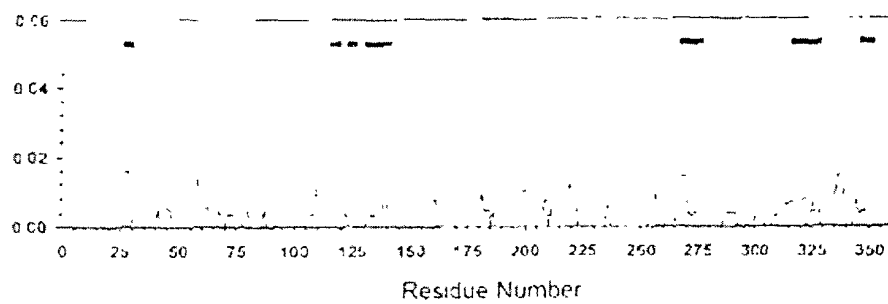


FIG. 4C

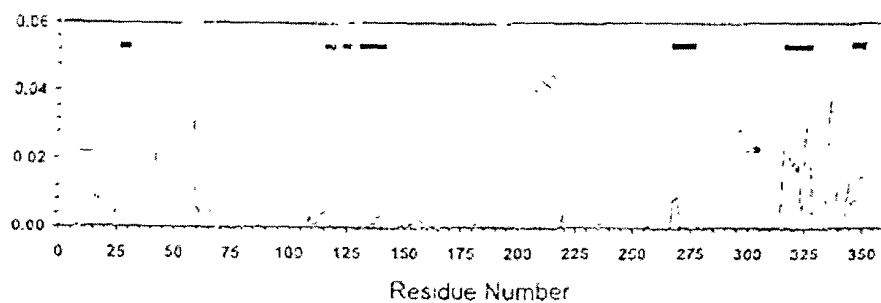


FIG. 4D

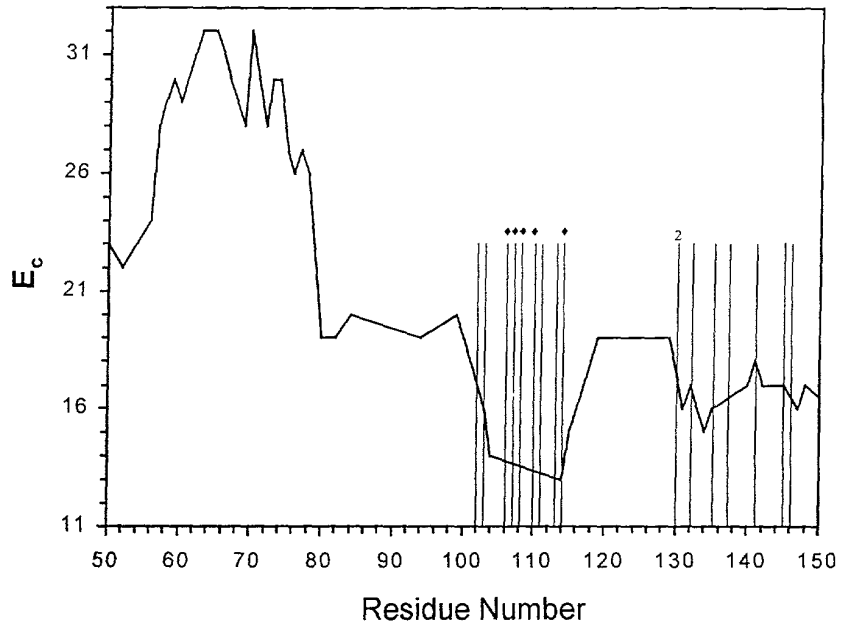


FIG. 5

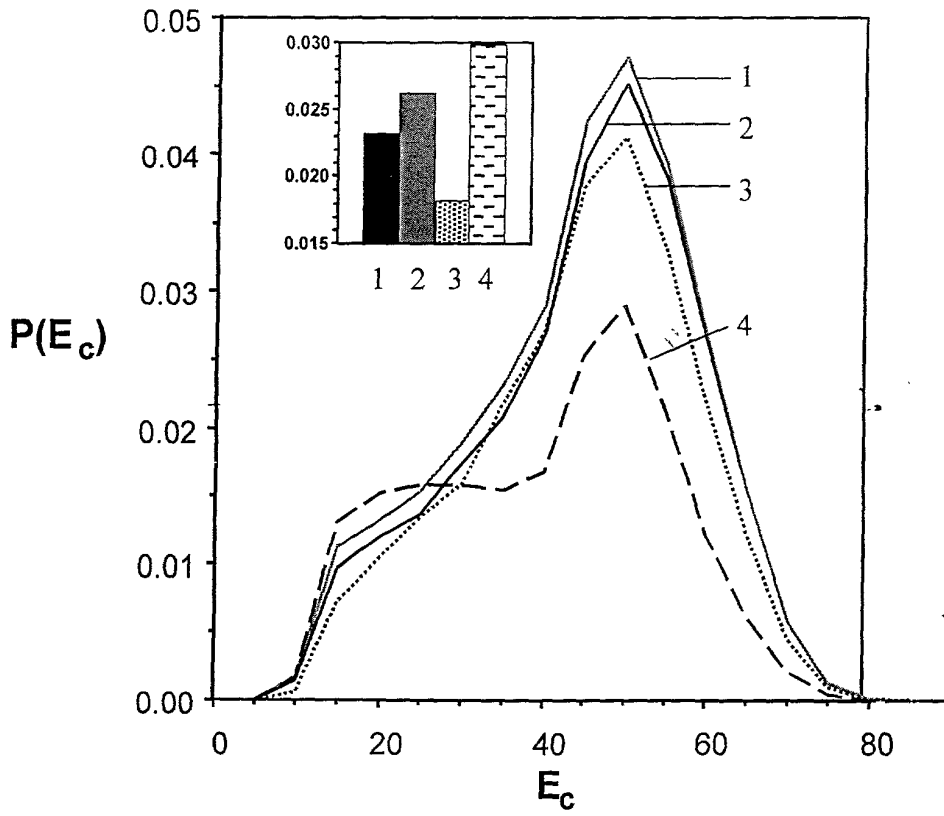


FIG. 6

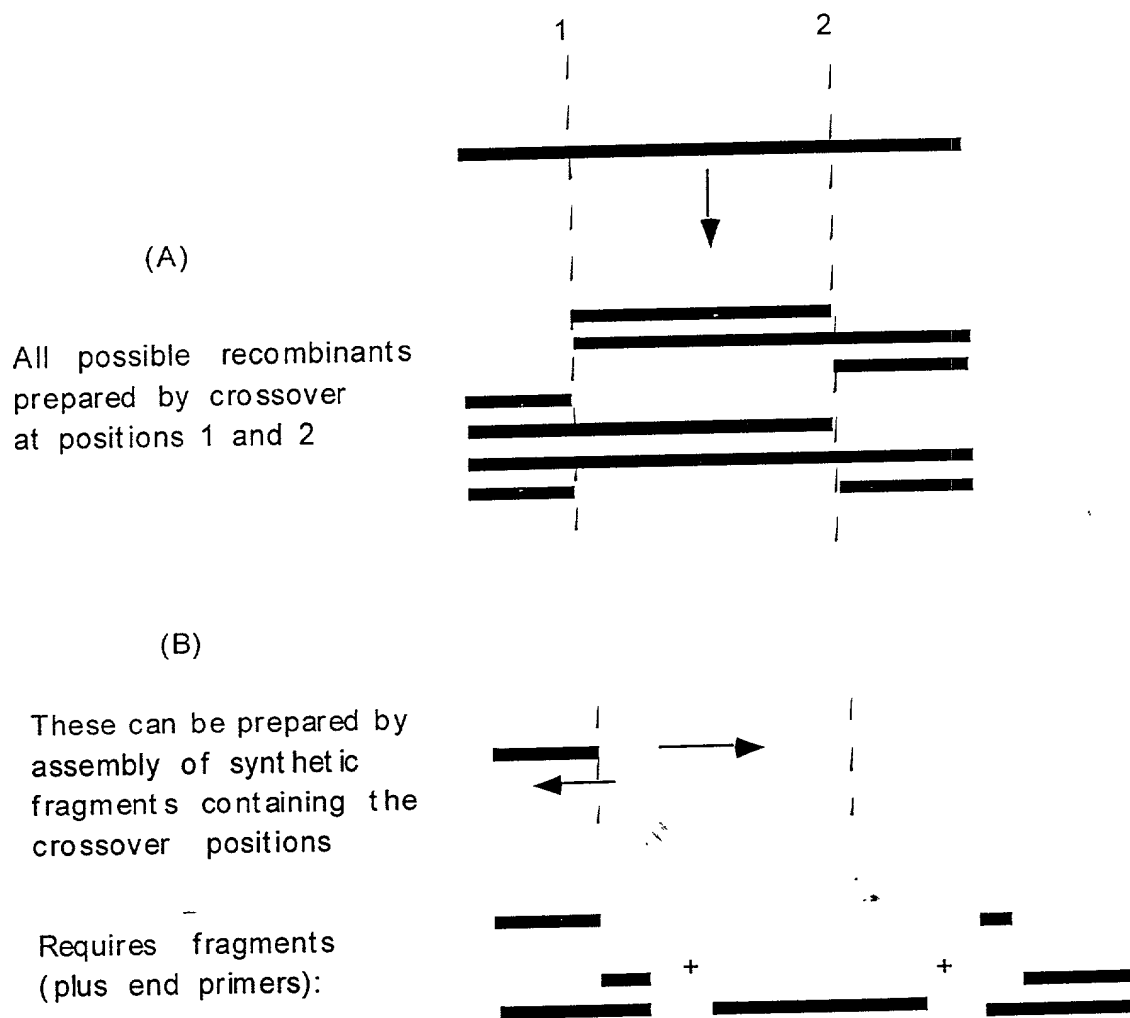
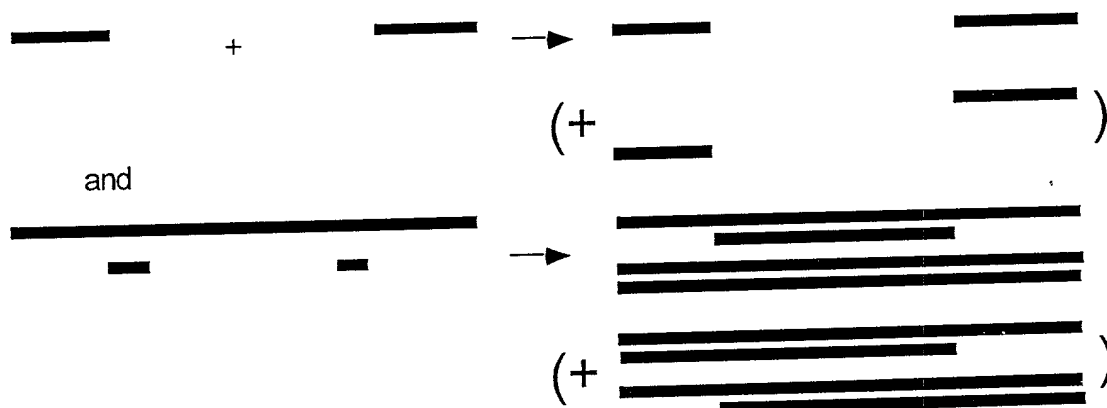


FIG. 7



Extension of synthetic  
fragments against a  
parent template strand  
and gap repair

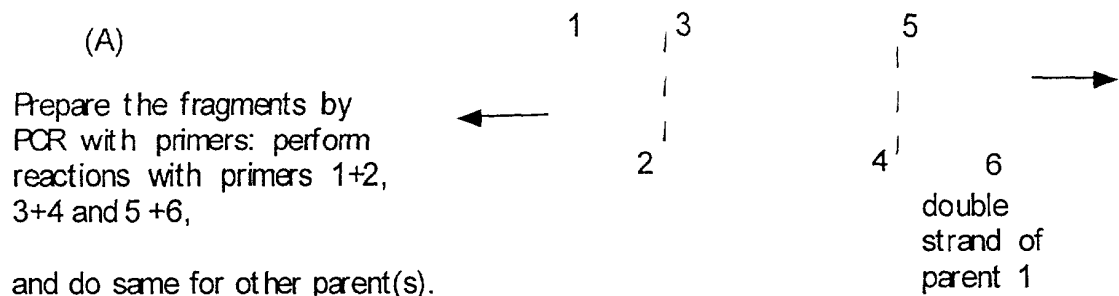


heteroduplex recombination  
(remove parent homoduplexes)

library of recombinants  
with crossovers in regions  
of non-identity

FIG. 8

10016658 102601



(B)

Reassemble fragments in a pool, by PCR with 1+ 6

FIG. 9

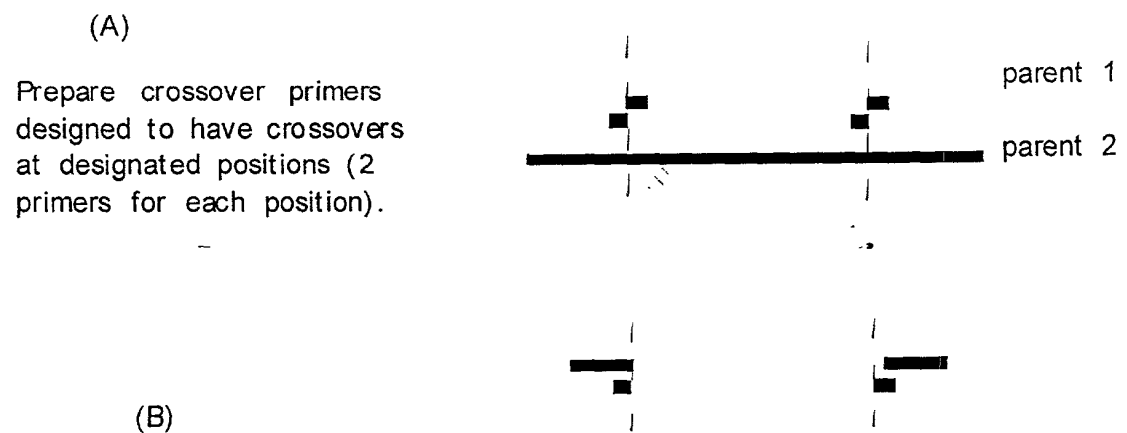


FIG. 10

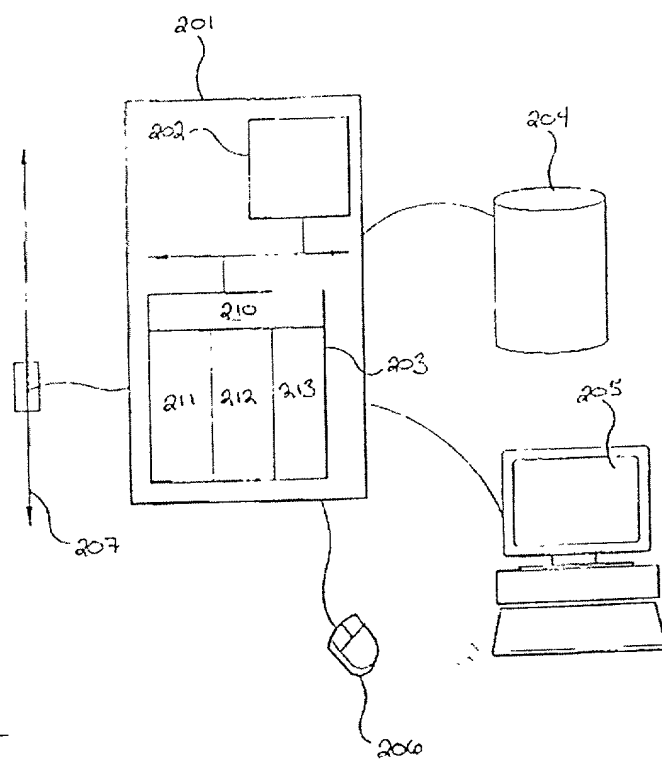
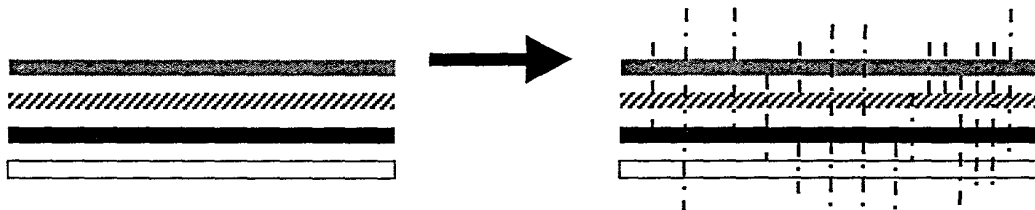


FIG. 11

[illegible]

2. Determine all possible crossover points according to sequence identity algorithm



- 

5. Pick random number, if less than  $p$ , copy random new parent until next cut point is reached.

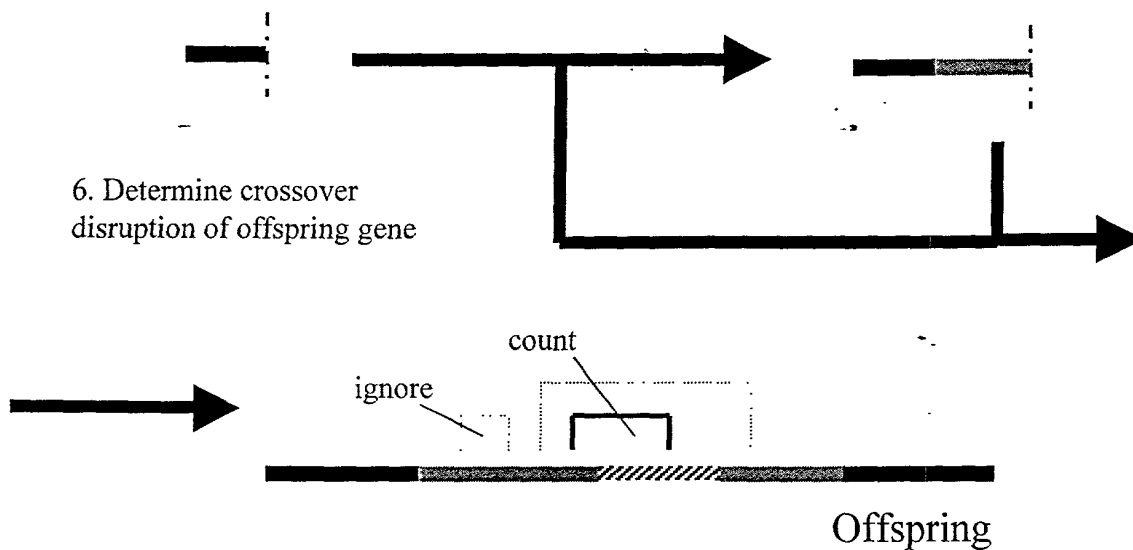


FIG. 12

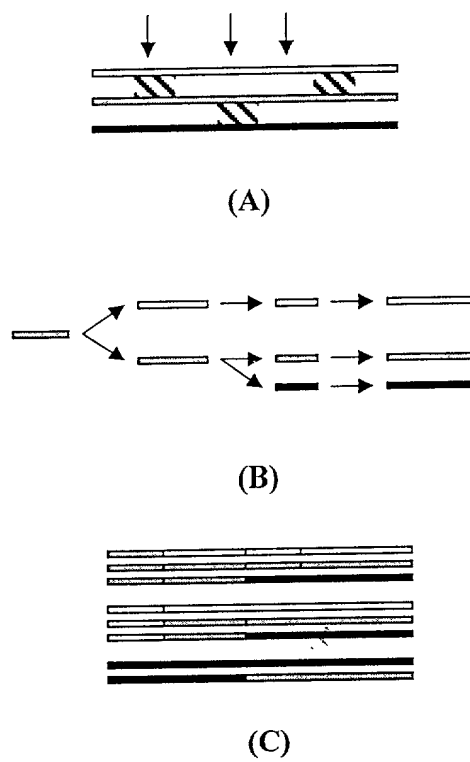
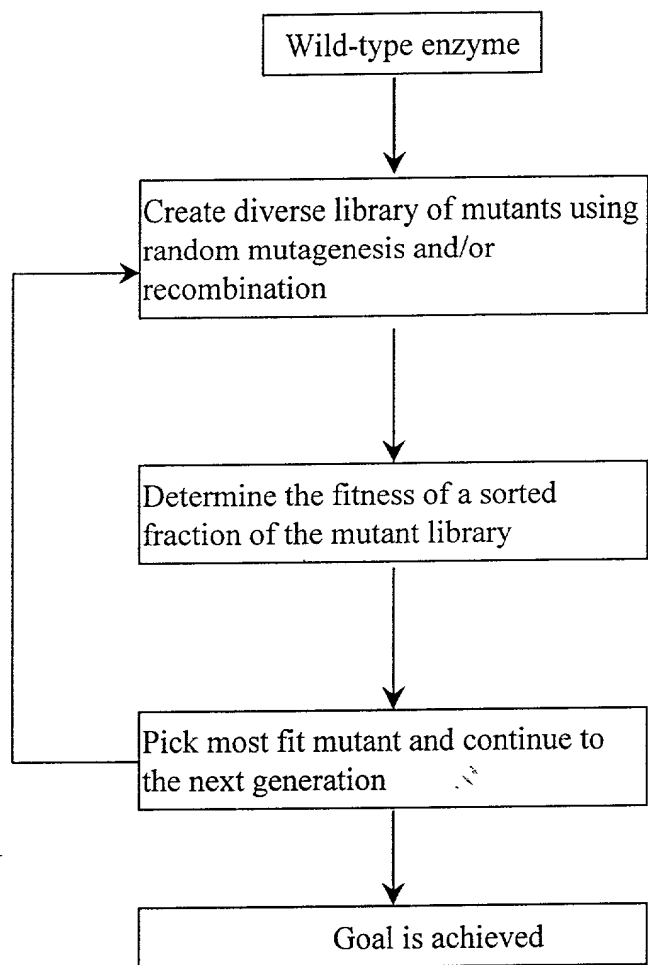


FIG. 13

# DIRECTED EVOLUTION ALGORITHM



**FIG. 14**

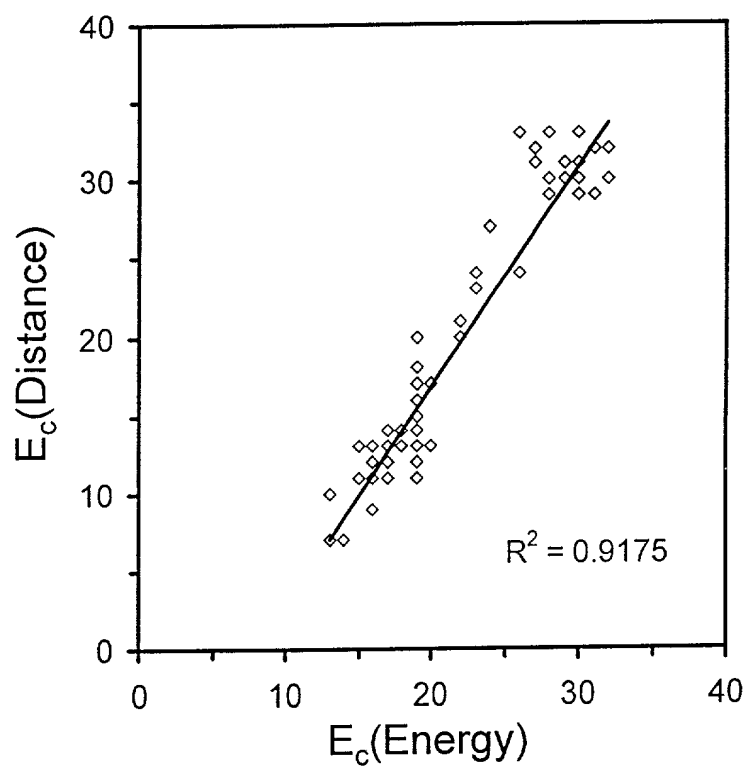


FIG. 15

1004668-103601

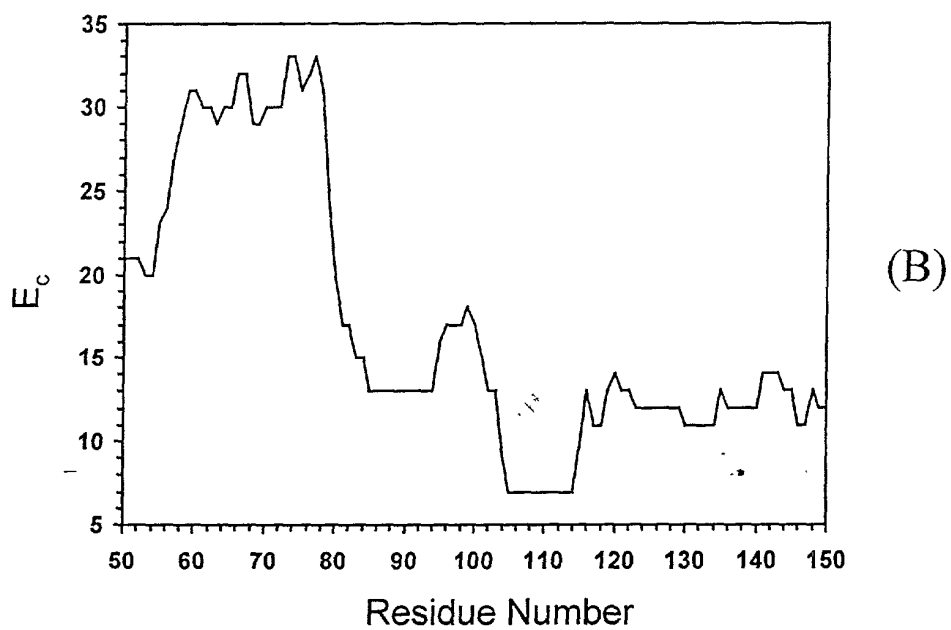
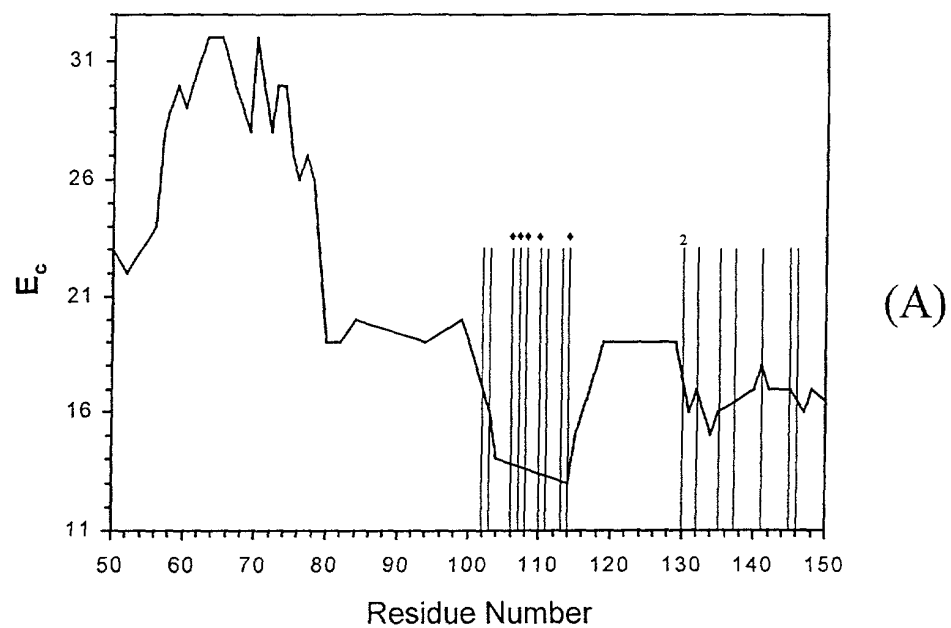


FIG. 16





(A)

Experimental Data:

	wt	wt-insert	1	2
Tm(dC)	52	55.2	n.d.	54.3
Tm(dC)	49.5	53.3	44.5	52.5
t1/2	12.1	2586	-	87.5
t1/2	53	138	4	308

(B)

Calculations:

	All schema		Fragments		Z-score	
	av	stdev	1	2	1	2
Ec	19.260	4.090	10.770	8.124	-2.076	-2.723
Ec*	0.006	0.002	0.014	0.005	4.838	-0.857

**FIG. 17**

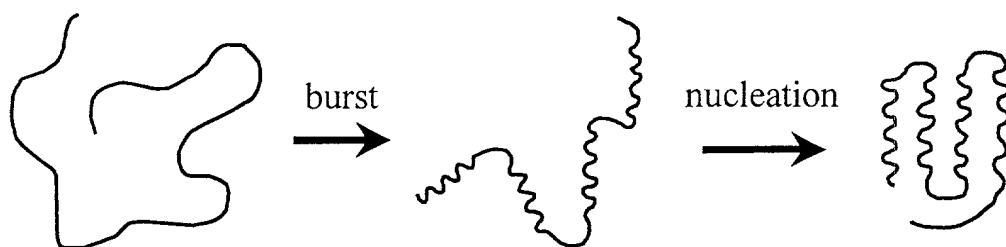



FIG. 18

The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, , folds an above average number of residues into a given sphere size, then it is compact.

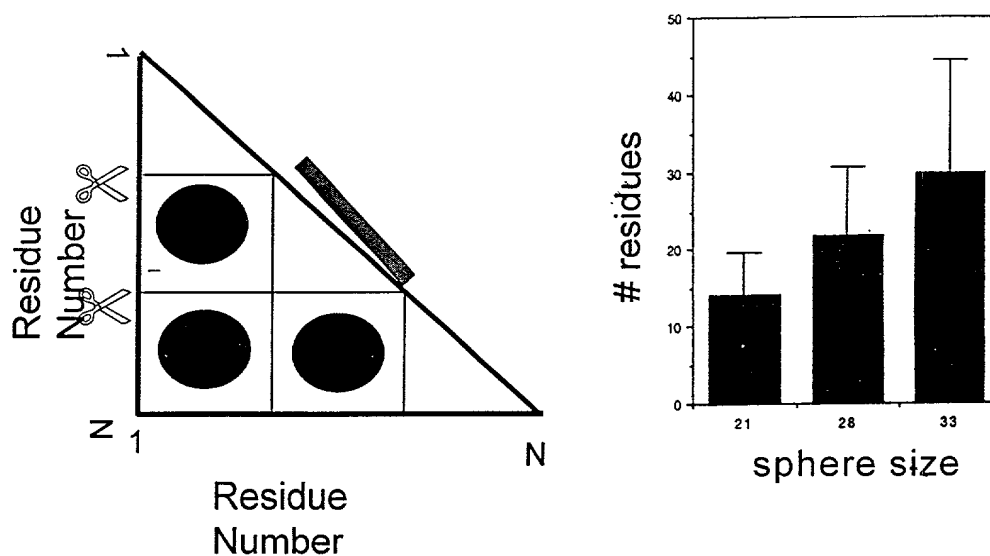


FIG. 19

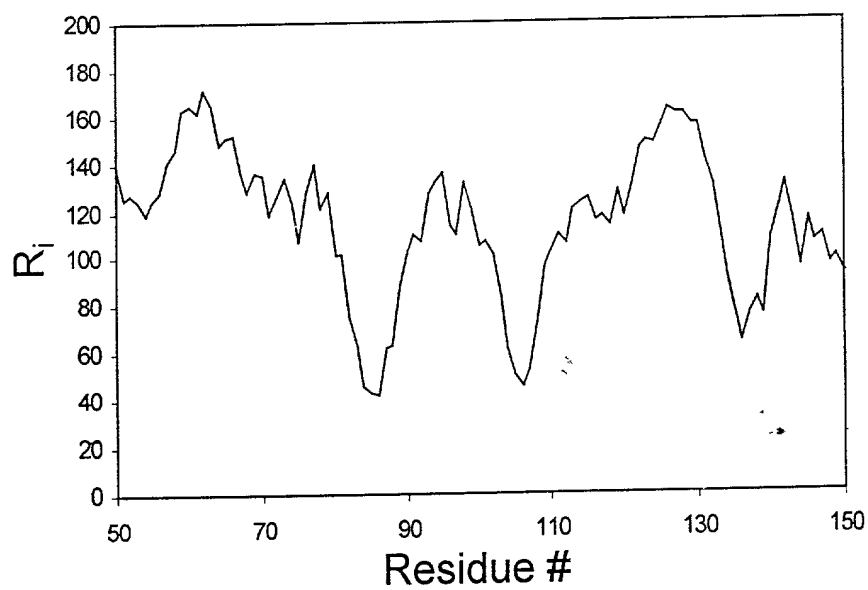
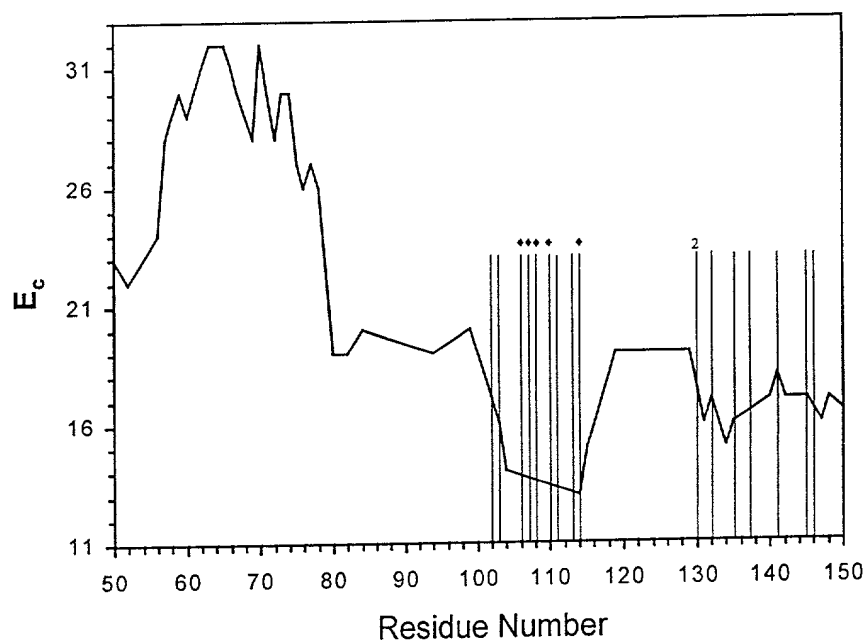


FIG. 20

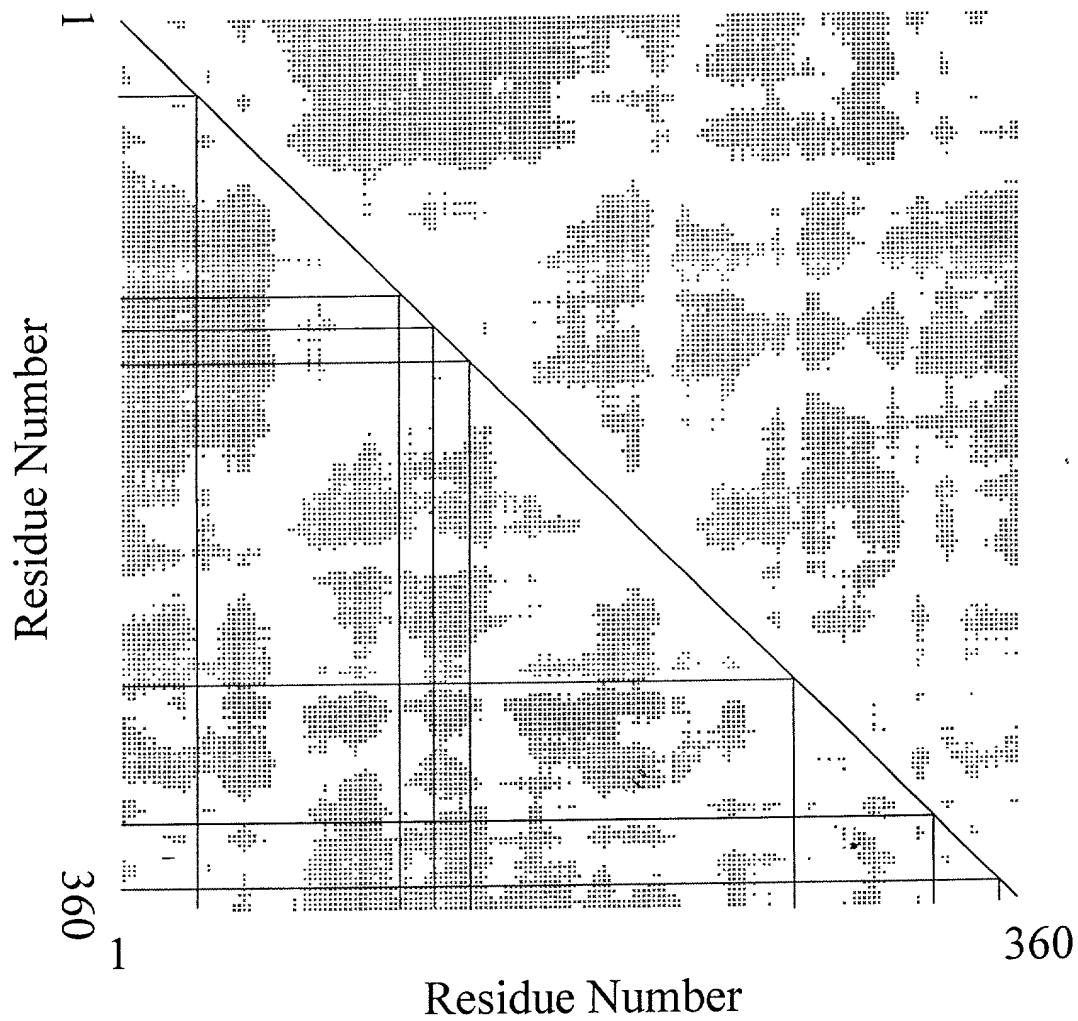


FIG. 21

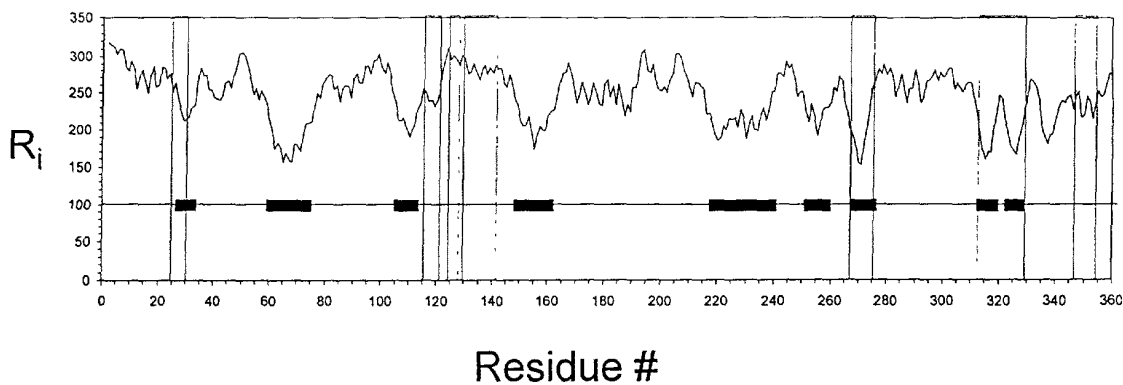
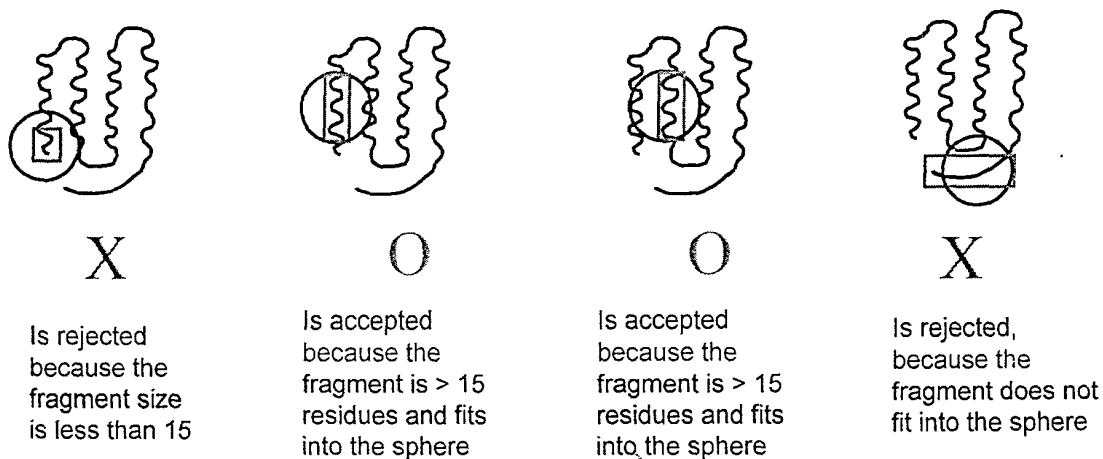


FIG. 22



(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments at least the average number of residues for that sphere size or greater (e.g., >15 for 21 angstrom sphere); (3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; (4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

FIG. 23

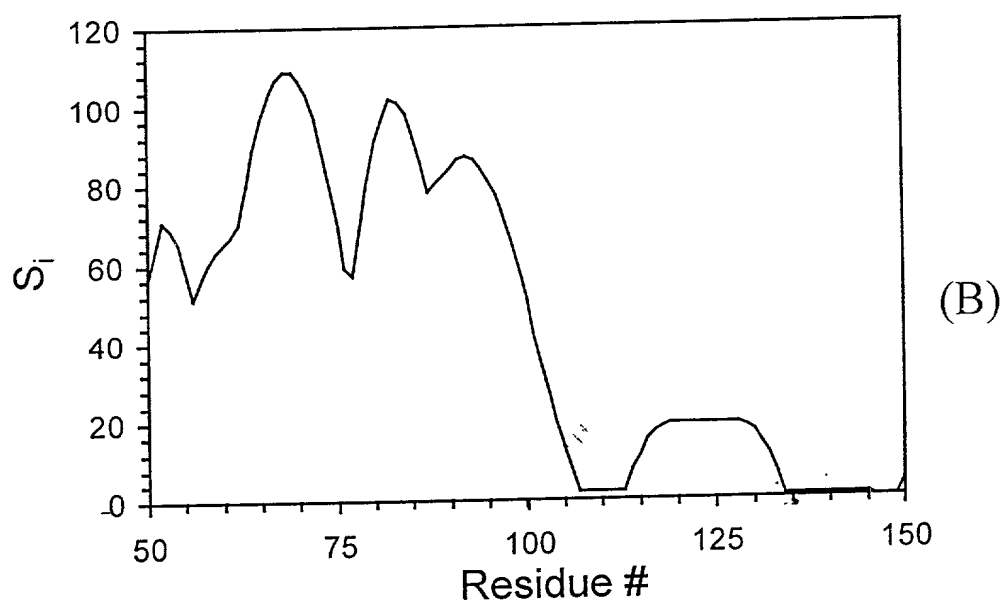
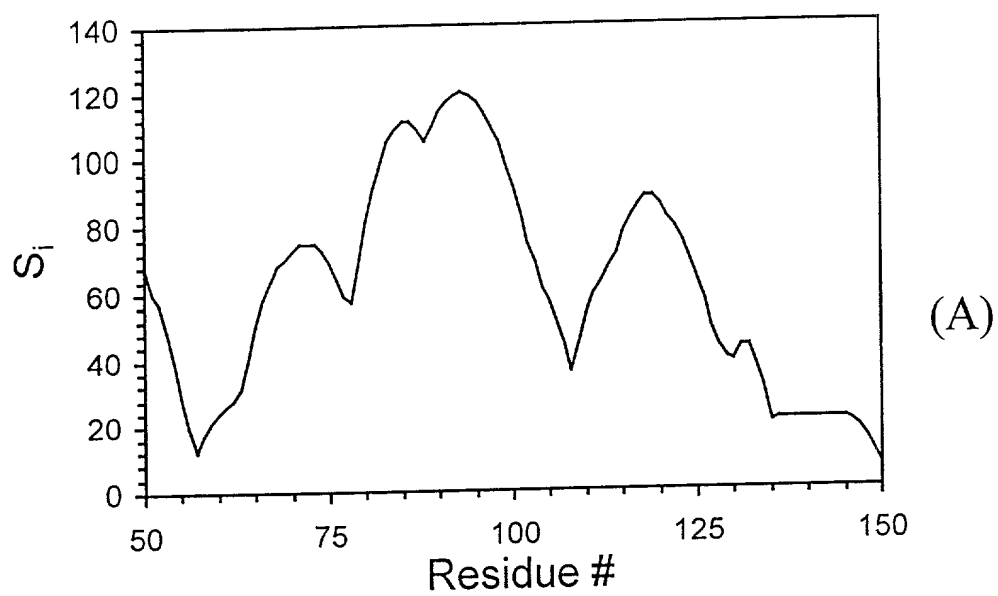


FIG. 24

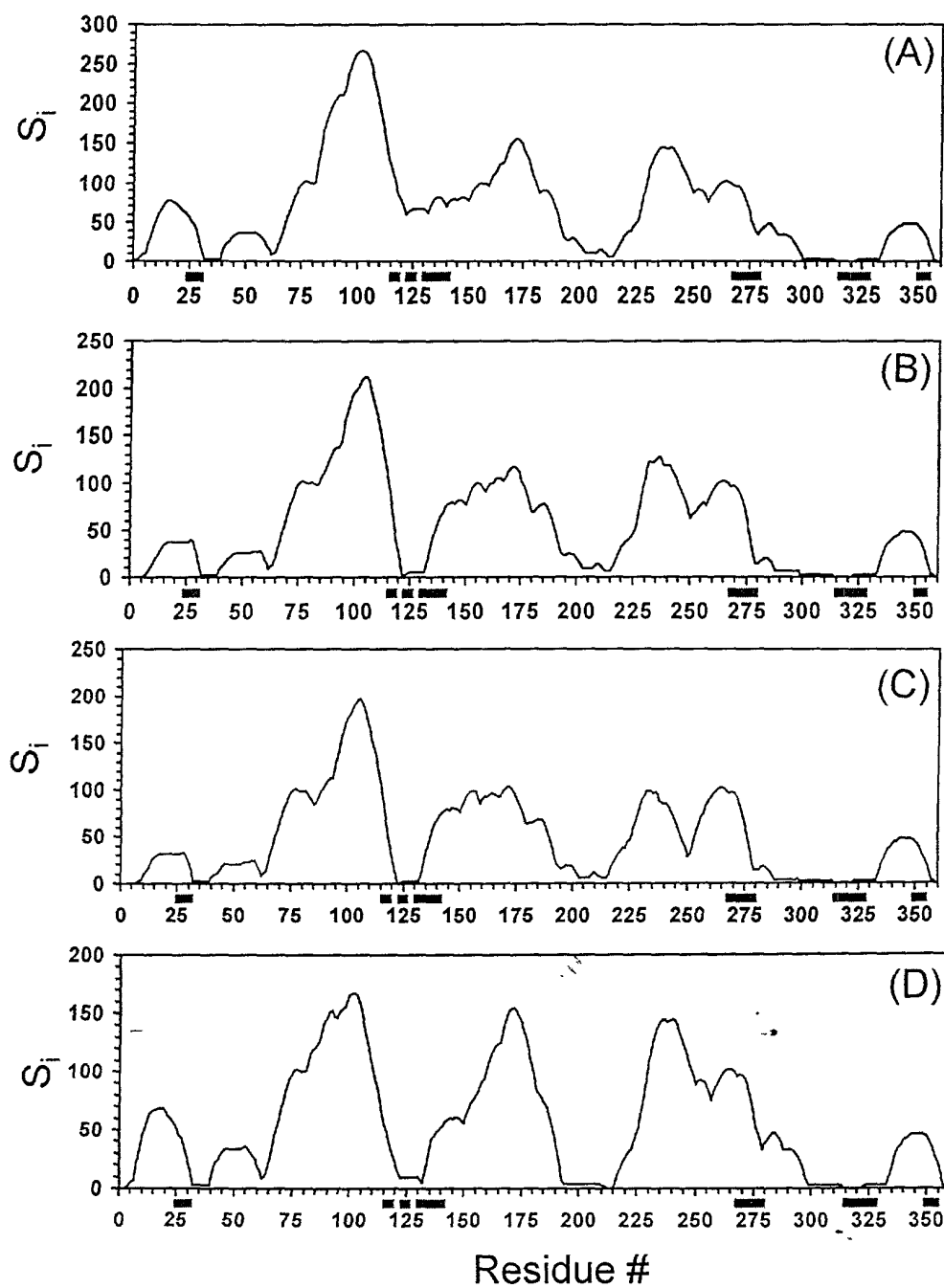


FIG. 25

1001668-102601

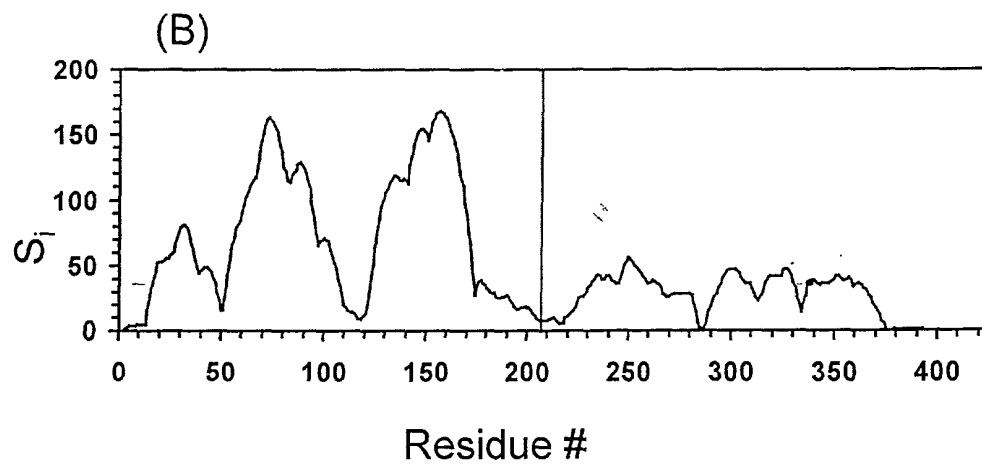
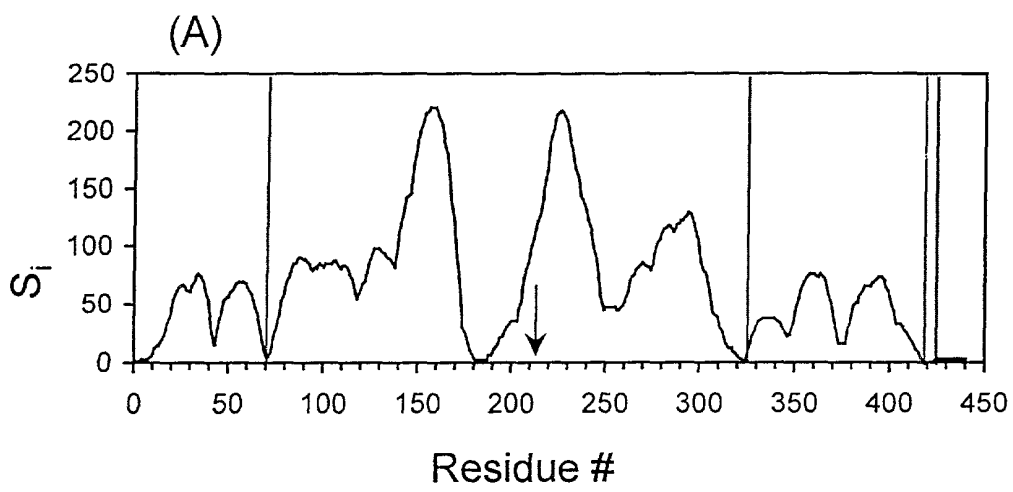


FIG. 26



1004668-1060  
"SECRET" 890900T

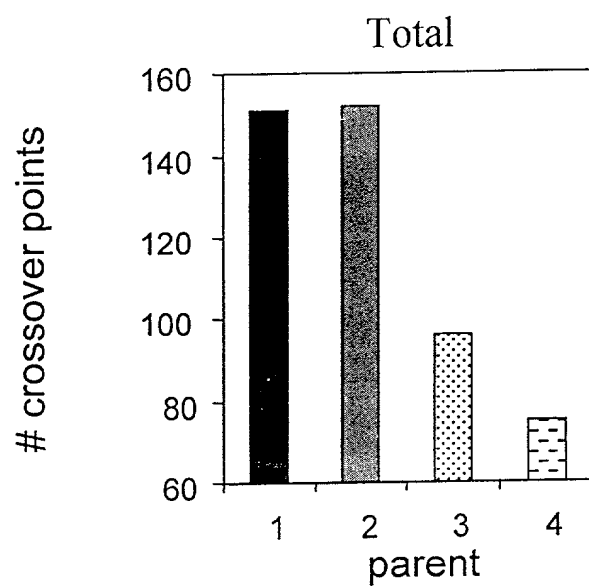


FIG. 27A

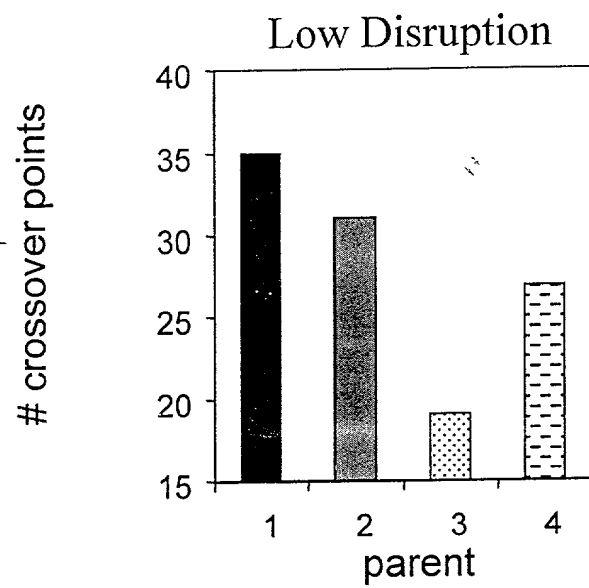


FIG. 27B

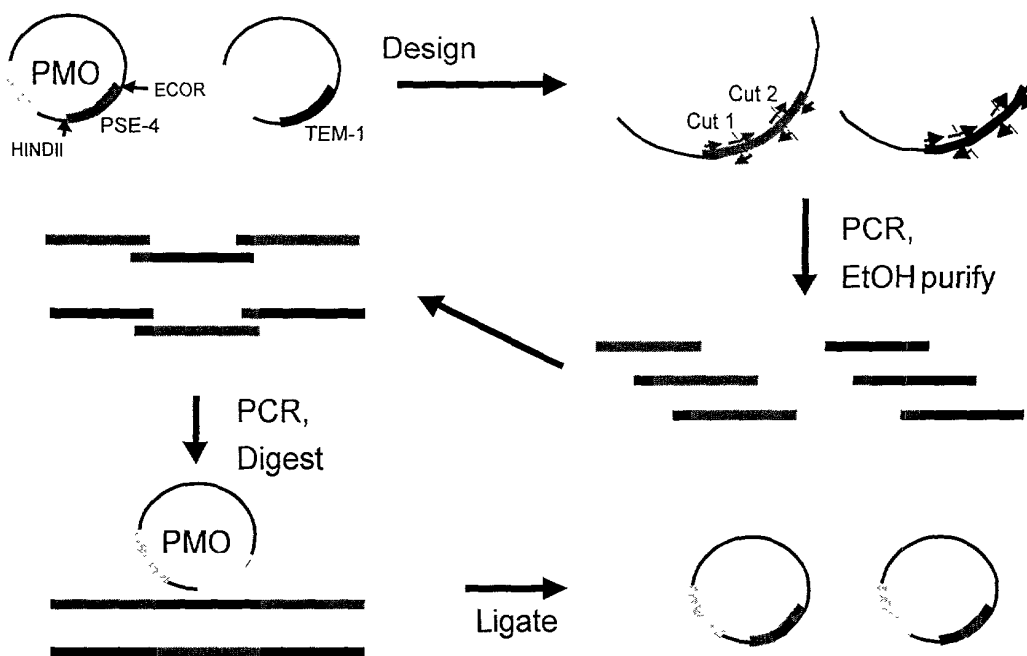


FIG. 28

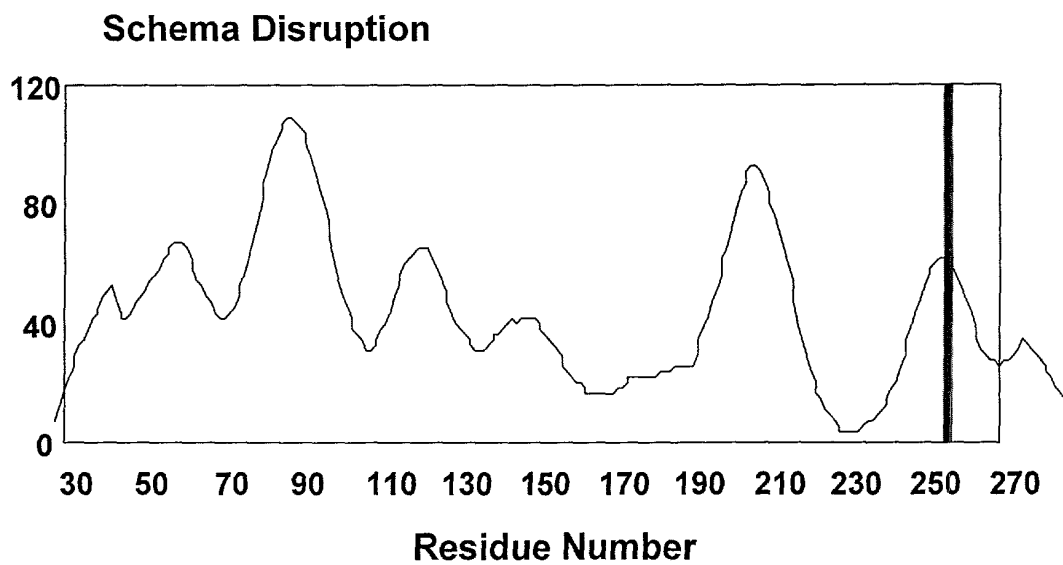


FIG. 29

1001666-102604

Schema Disruption

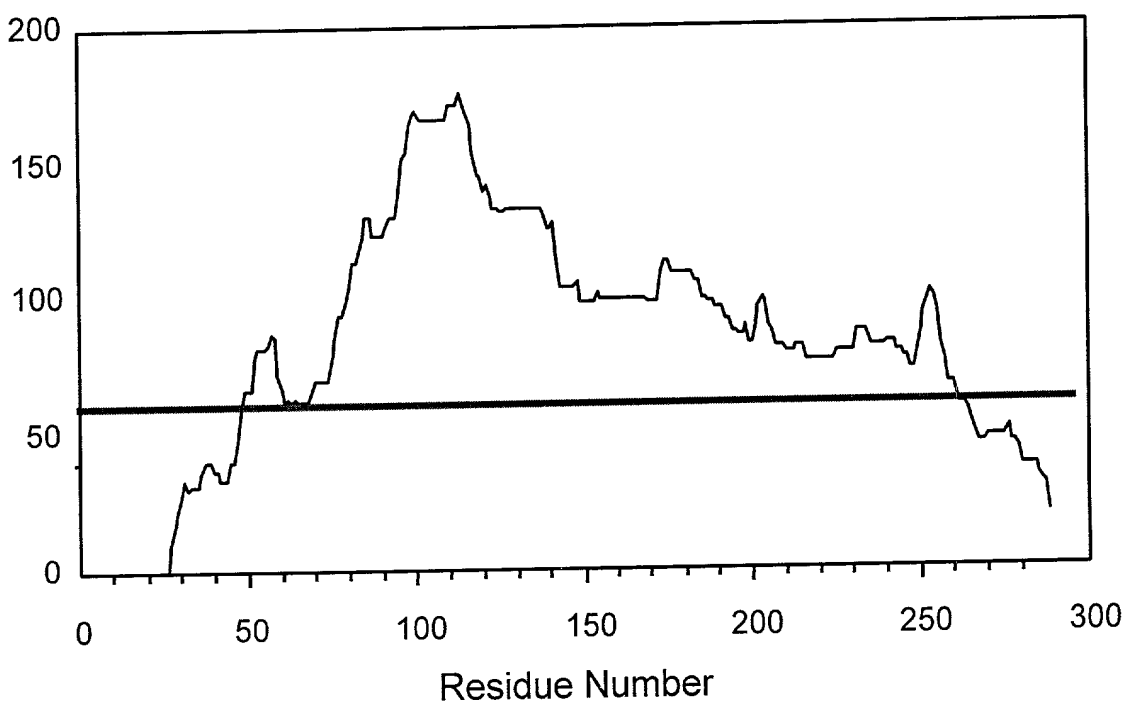


FIG. 30

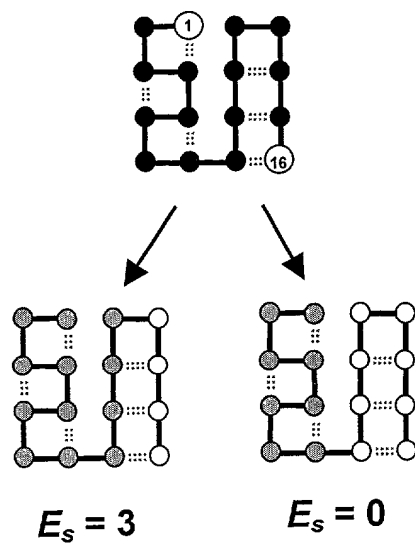


FIG. 31A

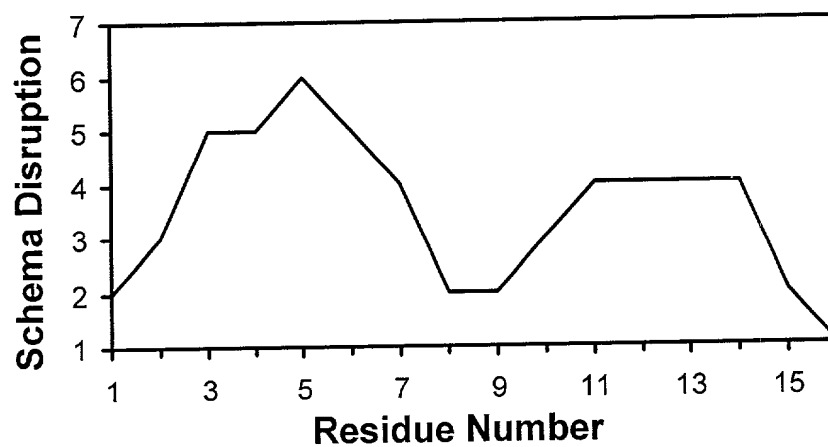


FIG. 31B

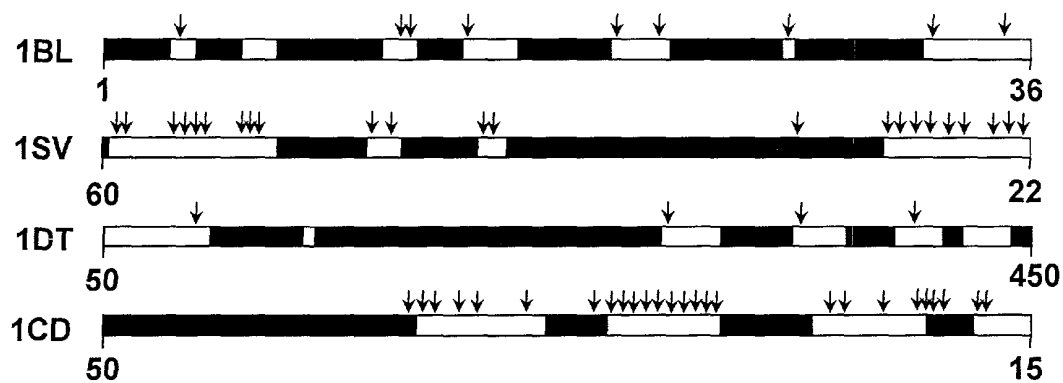


FIG. 32

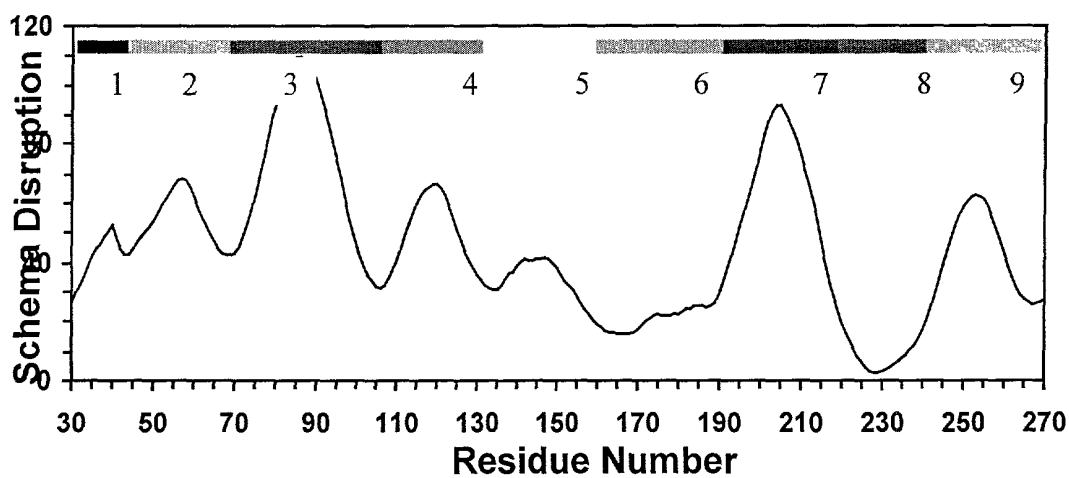


FIG. 33

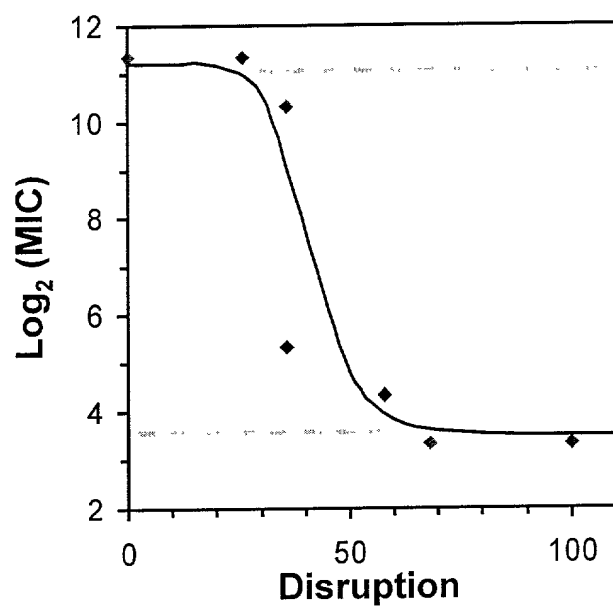


FIG. 34